Claims:

1. A chaperone polypeptide having an amino acid sequence selected from at least amino acid residues 230-271 but no more than residues 150-455 or 151-456 of a GroEL sequence substantially as shown in Figure 7, or a corresponding sequence of a substantially homologous chaperone polypeptide, or a modified, mutated or variant sequence thereof having chaperone activity.

2. Monomeric polypeptide having chaperone activity and incapable of multimerisation in solution.

3. A chaperone polypeptide which, when in solution, remains monomeric and has the ability to refold, reactivate or recondition proteins, said polypeptide including the protein binding active site motif:

1 X X X 2 X X 3 4 X X 5 X X X X X X X X X X X X X X X X X 6 X X 7
8 X 9 10 11 X X 12 X 13 14

wherein 1 is selected from amino acid residues:

I, M, L, V, S, F or A;

wherein 2 is selected from: L, I, P, V or A;
wherein 3 is selected from: L, E, V, H or I;
wherein 4 is selected from: E, A, R, L, Q, or N;
wherein 5 is selected from: A, V, I, M, L, N, S, R, T, Q or K;
wherein 6 is selected from: E, D or G;
wherein 7 is selected from: A, P, S, T, G or L;
wherein 8 is selected from: T, A, N, S or V;
wherein 9 is selected from: V, L, I or A;
wherein 10 is selected from: V, L, I, F or H;
wherein 11 is selected from: N, S or L;

wherein 12 is selected from: R, K, N, Q, L or S;

wherein 13 is selected from: I, T, S, G, V, A, Q, N, K, F or P;

wherein 14 is selected from: V, I, L, F, D or T; and

wherein the Xs represent a peptide bond or bonds or at least one amino acid residue,

or a functional variant thereof in which one or more of the numbered amino acid residues 1 to 14 has undergone a conservative substitution.

4. A chaperone polypeptide which, when in solution, remains monomeric and has the ability to refold, reactivate or recondition proteins, said polypeptide including at least one protein binding active site motif moiety selected from:

(a) 1 X X X 2 X X 3 4 X and

(b) X 5 X X X X X X X X X X X X X X X X X 6 X X 7 8 X 9 10 11 X X 12 X 13 14

wherein 1 is selected from amino acid residues:

I, M, L, V, S, F or A;

wherein 2 is selected from: L, I, P, V or A;

wherein 3 is selected from: L, E, V, H or I;

wherein 4 is selected from: E, A, R, L, Q, or N;

wherein 5 is selected from: A, V, I, M, L, N, S, R, T, Q or K;

wherein 6 is selected from: E, D or G;

wherein 7 is selected from: A, P, S, T, G or L;

wherein 8 is selected from: T, A, N, S or V;

wherein 9 is selected from: V, L, I or A;

wherein 10 is selected from: V, L, I, F or H;

wherein 11 is selected from: N, S or L;

wherein 12 is selected from: R, K, N, Q, L or S;

wherein 13 is selected from: I, T, S, G, V, A, Q, N, K, F or P;

wherein 14 is selected from: V, I, L, F, D or T; and

wherein X is at least one amino acid residue,

or a functional variant thereof in which one or more of the numbered amino acid residues 1 to 14 has undergone a conservative substitution.

5. A chaperone polypeptide which, when in solution, remains monomeric and has the ability to refold, reactivate or recondition proteins, said polypeptide including the protein binding active site motif:

IXXXLXXLEXXAXXXXXXXXXXXXXXXXEXX
ATXVVNXXRXIV

wherein X is at least one amino acid residue, or a functional variant thereof in which one or more of the specified amino acid residues has undergone a conservative substitution.

6. A chaperone polypeptide which, when in solution, remains monomeric and has the ability to refold, reactivate or recondition proteins, said polypeptide including at least one protein binding active site motif moiety selected from:

(a) IXXXLXXLEX

(b) XAXXXXXXXXXXXXXXXXEXXATXVV
NXXRXIV

wherein X is at least one amino acid residue, or a functional variant thereof in which one or more of the specified amino acid residues has undergone a conservative substitution.

7. A chaperone polypeptide as claimed in any one of claims 3 to 6 in which the active site motif or an active site motif moiety includes the conserved sequence:

PLL(V)I(V)IA(S)EDV(I)EGEAL

in which amino acid symbols in parenthesis are alternatives to the immediately preceding symbol reading left to right.

8. Monomeric polypeptide having chaperone activity and incapable of multimerisation characterised in that in the absence of ATP the polypeptide has a protein refolding activity of more than 50%, preferably 60%, even more preferably 75%, said refolding activity being determined by contacting the polypeptide with an inactivated protein of known specific activity prior to inactivation, and then determining the specific activity of the said protein after contact with the polypeptide, the % refolding activity being:

$$\frac{\text{specific activity of protein after contact with polypeptide} \times 100}{\text{specific activity of protein prior to inactivation} \quad 1}$$

9. A polypeptide as claimed in ~~any preceding claim,~~ claim 1 wherein the chaperone activity is determined by the refolding of cyclophilin A.

10. A polypeptide as claimed in claim 9 wherein 8M urea denatured cyclophilin A (100μM) is diluted into 100mM potassium phosphate buffer pH7.0, 10mM DTT to a final concentration of 1μM and then contacted with at least 1μM of said polypeptide at 25°C for at least 5 min, the resultant cyclophilin A activity being

assayed by the method of Fischer G *et al* (1984) Biomed Biochim Acta 43: 1101-1111.

11. A polypeptide as claimed ~~in any preceding claim~~ being an hsp60 polypeptide, ~~preferably~~ a GroEL polypeptide.

12. A polypeptide ~~as claimed in any preceding claim~~ which comprises at least an amino acid sequence selected from GroEL residues:

(a) 191-329, 191-330, 191-331, 191-332, 191-333, 191-334, 191-335, 191-336, 191-337, 191-338, 191-339, 191-340, 191-341, 191-342, 191-343, 191-344, 191-345, 191-346, 191-347, 191-348, 191-349, 191-350, 191-351, 191-352, 191-353, 191-354, 191-355, 191-356, 191-357, 191-358, 191-359, 191-360, 191-361, 191-362, 191-363, 191-364, 191-365, 191-366, 191-367, 191-368, 191-369, 191-370, 191-371, 191-372, 191-373, 191-374, 191-375 or 191-376, or

(b) 192-329, 192-330, 192-331, 192-332, 192-333, 192-334, 192-335, 192-336, 192-337, 192-338, 192-339, 192-340, 192-341, 192-342, 192-343, 192-344, 192-345. 192-346, 192-347, 192-348, 192-349, 192-350, 192-351, 192-352, 192-353, 192-354, 192-355, 192-356, 192-357, 192-358, 192-359, 192-360, 192-361, 192-362, 192-363, 192-364, 192-365, 192-366, 192-367, 192-368, 192-369, 192-370, 192-371, 192-372, 192-373, 192-374, 192-375 or 192-376, or

(c) 193-329, 193-330, 193-331, 193-332, 193-333, 193-334, 193-335, 193-336, 193-337, 193-338, 193-339, 193-340, 193-341, 193-342, 193-343, 193-344, 193-345, 193-346, 193-347, 193-348, 193-349, 193-350, 193-351, 193-352, 193-353, 193-354, 193-355, 193-356, 193-357, 193-358, 193-359, 193-360, 193-361, 193-362, 193-363, 193-364, 193-365, 193-366, 193-367, 193-368,

193-369, 193-370, 193-371, 193-372, 193-373, 193-374, 193-375 or 193-376, or

(d) 230-271, 229-271, 229-272, 228-272, 228-273, ...*et seq*... 194-328, 194-329, or

the equivalent residues of substantially homologous chaperonins, or a modified, mutated or variant sequence thereof.

13. A polypeptide as claimed in claim 8, wherein the selected amino acid sequence is selected from the group consiting of 230-271, 191-345, 191-376, 193-335 and 193-337 of GroEL, the equivalent residues of substantially homologous chaperonins, and a modified, mutated or variant sequence thereof.

14. A polypeptide as claimed ~~in any preceding claim~~ further comprising a polyamino acid sequence, ~~preferably~~ an N-terminal polyamino acid sequence.

15. A polypeptide as claim in claim 14, wherein the polyamino acid sequence is a polyhistidine sequence.

16. A polypeptide as claimed in claim 14 or ~~claim 15,~~ wherein the polyamino acid sequence includes a cleavage site cleavable by a cleavage agent, ~~preferably~~ said cleavage agent is thrombin.

17. A polypeptide as claimed ~~in any one of claims~~ 14 ~~to 16~~ wherein the further polyamino acid sequence comprises a number of amino acid residues in the range 2 to 500, preferably 5 to 100, more ~~preferably~~ 17 to 39.

18. A polypeptide as claimed ~~in any preceding claim and is~~ immobilised form, optionally immobilised to a chromatographic matrix, ~~preferably~~ an agarose resin.

19. A polypeptide as claimed in claim 18, wherein the agarose resin is a nickel-nitrilo-tri-acetic acid (NTA)-ligated agarose resin.

20. A polypeptide as claimed ~~in any preceding claim~~ claim 1 fused to a heterologous protein or polypeptide.

21. A recombinant polypeptide as claimed ~~in any preceding claim~~ claim

22. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide as defined in ~~any one of claims 1 to 21~~ claim or a nucleotide sequence hybridisable thereto and optionally encoding a polypeptide having chaperone activity.

23. A recombinant nucleic acid molecule for use in cloning and/or expressing a nucleic acid sequence, said recombinant nucleic acid molecule comprising:

(a) a nucleotide sequence encoding amino acid residues 191-376 of GroEL, or

(b) a nucleotide sequence encoding amino acid residues 191-345 of GroEL, or

(c) a nucleotide sequence encoding amino acid residues 193-337of Gro EL, or

(d) a nucleotide sequence encoding amino acid residues 193-335of Gro EL, or

(e) a nucleotide sequence encoding amino acid residues of GroEL selected from amino acid residues:

(i) 191-329, 191-330, 191-331, 191-332, 191-333, 191-334, 191-335, 191-336, 191-337, 191-338, 191-339, 191-340, 191-341, 191-342, 191-343, 191-344,

191-345, 191-346, 191-347, 191-348, 191-349, 191-350, 191-351, 191-352, 191-353, 191-354, 191-355, 191-356, 191-357, 191-358, 191-359, 191-360, 191-361, 191-362, 191-363, 191-364, 191-365, 191-366, 191-367, 191-368, 191-369, 191-370, 191-371, 191-372, 191-373, 191-374, 191-375 or 191-376, or

(ii) 192-329, 192-330, 192-331, 192-332, 192-333, 192-334, 192-335, 192-336, 192-337, 192-338, 192-339, 192-340, 192-341, 192-342, 192-343, 192-344, 192-345, 192-346, 192-347, 192-348, 192-349, 192-350, 192-351, 192-352, 192-353, 192-354, 192-355, 192-356, 192-357, 192-358, 192-359, 192-360, 192-361, 192-362, 192-363, 192-364, 192-365, 192-366, 192-367, 192-368, 192-369, 192-370, 192-371, 192-372, 192-373, 192-374, 192-375 or 192-376, or

(iii) 193-329, 193-330, 193-331, 193-332, 193-333, 193-334, 193-335, 193-336, 193-337, 193-338, 193-339, 193-340, 193-341, 193-342, 193-343, 193-344, 193-345, 193-346, 193-347, 193-348, 193-349, 193-350, 193-351, 193-352, 193-353, 193-354, 193-355, 193-356, 193-357, 193-358, 193-359, 193-360, 193-361, 193-362, 193-363, 193-364, 193-365, 193-366, 193-367, 193-368, 193-369, 193-370, 193-371, 193-372, 193-373, 193-374, 193-375 or 193-376, or

(f) 230-271, 229-271, 229-272, 228-272, 228-273, ...*et seq*... 194-328, 194-329, or

(g) a nucleotide sequence hybridisable to any of (a), (b), (c), (d), (e) or (f) above and encoding a monomeric polypeptide having chaperone activity, or

(h) degenerate nucleotide sequences corresponding to (a), (b), (c), (d), (e), (f) or (g) above.

24. A vector comprising a nucleic acid as claimed in ~~claim 22 or~~ claim 23.

25. A host cell transformed with a nucleic acid as defined in ~~claim 22 or~~ claim 23 ~~or vector as defined in claim 24~~.

26. A method of making a polypeptide as defined ~~in any one of claims~~ claim 1 ~~to 21~~ comprising transforming a host cell with a nucleic acid encoding said polypeptide, culturing the transformed cell and expressing said polypeptide.

27. A method of making a polypeptide as claimed in claim 26 wherein the nucleic acid is as defined in claim 22 or claim 23.

28. A method as claimed in ~~claim 26 or~~ claim 27, wherein the expressed polypeptide product is subject to cleavage.

29. A pharmaceutical formulation comprising a polypeptide of ~~any one of claims~~ claim 1 ~~to 21~~, optionally together with a diluent, carrier or excipient.

30. A polypeptide as defined in ~~any one of claims~~ claim 1 ~~to 21~~ for use in the treatment of disease.

31. The use of a polypeptide as defined in ~~any one claims~~ claim 1 ~~to 21~~ in the manufacture of a medicament for the treatment of disease associated with protein/polypeptide structure.

32. A nucleic acid molecule as defined in ~~claims 22 or~~ claim 23 for use in the treatment of disease.

33. The use of nucleic acid molecule as defined in ~~claim 22 or~~ claim 23 in the manufacture of a medicament for the treatment of disease associated with protein/polypeptide structure.

34. A method of reconditioning a molecule preferably a protein comprising contacting said protein with a polypeptide of ~~any one of claims~~ claim 1 ~~to 21~~.

35. A method as claimed in claim 34, wherein the protein is subjected to inactivation or denaturation prior to contacting with said polypeptide.

36. A method as claimed in ~~claim 34 or~~ claim 35, wherein the polypeptide is immobilised to a solid phase.

37. A method as claimed in ~~claim 33 or~~ claim 34, wherein the polypeptide is immobilised to a solid phase, preferably a chromatographic matrix, and the contacting of protein and polypeptide is carried out by applying the protein to the top of a bed of the matrix packed in a column and then eluting the polypeptide through the column.

38. Use of a polypeptide as claimed in ~~any one of claims 1 to 21~~ claim 1 for altering the structure of a molecule.

39. The use of claim 38, wherein the molecule is a protein or polypeptide and the alteration in structure is by folding unfolding or refolding.

40. The use of ~~claim 38 or~~ claim 39, wherein the stoichiometry between the polypeptide and the molecule being altered is about 1:1.

41. Use of a polypeptide as defined in ~~any one of claims 1 to 21~~ claim 1 in the purification or increase in yield, specific activity or quality of biological molecules, preferably said polypeptide being attached to a support.

42. A kit for reconditioning or refolding a molecule, preferably a protein, comprising a polypeptide of ~~any one of claims~~ claim 1 ~~to 21~~ immobilised to a solid phase and a container for holding said solid phase polypeptide.

43. Use of a polypeptide as defined in ~~any one of claims~~ claim 1 ~~to 21~~ in the production of a protein or polypeptide by recombinant means, wherein the said polypeptide is co-expressed with the protein or polypeptide thereby to improve the yield or quality of the protein or polypeptide.

44. An antibody reactive against a polypeptide as defined in ~~any one of claims 1 to 21.~~ claim 1

45. An antibody as claimed in claim 44 for use in the treatment of disease.

46. The use of an antibody as claimed in claim 44 in the manufacture of a medicament for the treatment of disease associated with protein/polypeptide structure.

47. A method of treating disease in which an effective amount of a polypeptide of any one of claims 1 to 21 is administered.

48. A method of treating disease which comprises administering an effective amount of an inhibitor of the chaperone activity of a polypeptide of ~~any one of claims 1 to 21.~~ claim 1

49. A method as claimed in claim 48 wherein said inhibitor is an antibody.

50. A method of treating disease by gene therapy which utilises a construct encoding a polypeptide of ~~any one of claims 1 to 20~~ claim n or an antagonist thereof.

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